JAMA Ophthalmology | Original Investigation

Genetic and Environmental Factors Associated With the Ganglion Cell Complex in a Healthy Aging British Cohort

Edward Bloch, MBBS; Ekaterina Yonova-Doing, MSc; Eneh Jones-Odeh, MBBS; Katie M. Williams, FRCOphth; Diana Kozareva, BSc; Christopher J. Hammond, MD

IMPORTANCE Measurement of ganglion cell complex (GCC) thickness may be more sensitive than current methods for glaucoma diagnosis and research. However, little is known about the factors influencing GCC thickness in the general population.

OBJECTIVES To investigate the heritability of and factors associated with GCC thickness in a healthy aging population.

DESIGN, SETTING, AND PARTICIPANTS A cross-sectional twin study was conducted from August 27, 2014, to March 31, 2016, among 1657 participants of white British ancestry from the TwinsUK study cohort without ocular pathologic conditions. Heritability analyses were conducted in 1432 twins (426 monozygous and 290 dizygous pairs). Association analyses were performed using univariable and multivariable stepwise linear regression models, taking family structure into account. Heritability analyses were conducted using maximum likelihood structural equation twin modeling.

MAIN OUTCOMES AND MEASURES Parameters measured included GCC thickness, autorefraction, intraocular pressure, blood pressure, body mass index, and cholesterol, creatinine, glucose, insulin, triglycerides, and urea levels. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease formula.

RESULTS Among the 1657 participants (mean [SD] age, 56.0 [15.3] years; 89.5% women and 10.5% men), the mean [SD] inner GCC thickness was 96.0 [7.6] μm (95% CI, 95.1-96.2). In multivariable modeling, the mean inner GCC thickness was associated with advancing age (β, −0.14; $P < .001$), increased body mass index (β, −0.15; $P = .001$), spherical equivalent (β, 0.70; $P < .001$), and higher estimated glomerular filtration rate (β, 0.03; $P = .02$). A 1-U increase in age or body mass index was associated with a 0.14-μm and 0.15-μm decrease in GCC thickness, respectively ($P < .001$), while a 1-U increase in spherical equivalent or estimated glomerular filtration rate was associated with a 0.70-μm ($P < .001$) and 0.03-μm ($P = .02$) increase in GCC thickness, respectively. Ganglion cell complex thickness was not associated with sex, intraocular pressure, or diabetes. Age-adjusted GCC thickness was highly heritable, with additive genetic effects explaining 81% (95% CI, 78%-84%) of phenotypic variance and individual environmental factors explaining the remaining 19% (95% CI, 16%-22%).

CONCLUSIONS AND RELEVANCE Ganglion cell complex thickness appears to be highly heritable and further genetic analysis may help identify new biological pathways for glaucoma. The results suggest it may be important to account for age, body mass index, refractive error, and sex when using GCC thickness as a diagnostic tool. Replication of their results is required, as is further research to explain the association between renal function and GCC thickness.

JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2016.4486
Published online November 17, 2016.

Author Affiliations: Department of Twin Research and Genetic Epidemiology, King's College London, London, England (Bloch, Yonova-Doing, Jones-Odeh, Williams, Kozareva, Hammond); Department of Ophthalmology, King's College London, London, England (Williams, Hammond).

Corresponding Author: Christopher J. Hammond, MD, Department of Twin Research and Genetic Epidemiology, King's College London, Westminster Bridge Road, Third Floor Block D South Wing, St Thomas' Hospital, London SE1 7EH, England (chris.hammond@kcl.ac.uk).

Glaucoma is the second leading cause of blindness worldwide, affecting up to 3% of the global population older than 40 years, and is forecasted to affect 79.6 million people worldwide by 2020.[1,2] Glaucoma comprises a group of optic neuropathies characterized by progressive, irreversible visual field loss. Impaired retrograde neurotrophic transport causes dysfunction of retinal ganglion cells and, ultimately, apoptosis with axonal atrophy.[3-5]

As loss of retinal ganglion cells is not visible on ophthalmoscopy, glaucoma is traditionally detected using optic nerve examination and visual field tests. These tests have low sensitivity, are prone to interobserver variation, and detect changes that occur relatively late in the disease process, leading to a diagnostic delay of up to 10 years.[6-9] The advent of spectral domain-optical coherence tomography (SD-OCT) has afforded the opportunity to acquire noninvasive in vivo high-resolution segmentation of the inner retinal layers.[10-12] Since the macula contains up to 50% of retinal ganglion cells, it is very sensitive to early glaucomatous damage.[13] Spectral domain-optical coherence tomography measurements of the macular ganglion cell complex (GCC) thickness, comprising the ganglion cell layer, the inner plexiform layer, and the retinal nerve fiber layer (RNFL), correlate closely with histologic data.[14] Various studies have investigated the diagnostic ability and validity of SD-OCT parameters, concluding that both GCC and peripapillary RNFL thickness have high sensitivity, specificity, and positive predictive value for disease.[15-27]

It is well established that older age and higher intraocular pressure (IOP) are risk factors for primary open-angle glaucoma (POAG).[28] It also has been suggested that factors such as vascular dysregulation[29-32] and diabetes[33] may play an important role in the development of POAG. In addition, family history is known to contribute to the risk of developing POAG[28] and genetic variants have been identified that play a role in POAG and its endophenotypes.[34,35]

The extent to which any of these factors may influence GCC thickness in a healthy population is unclear. We undertook a cross-sectional observational study to explore the systemic and ophthalmic factors influencing GCC thickness in a healthy population of European ancestry. In particular, we focused on parameters that might be associated with microvascular damage, such as blood pressure, diabetes, and renal function. To investigate to what extent genetic factors influence GCC thickness, we used a twin model design to perform a heritability study.

Key Points

Question What are the genetic and environmental factors associated with ganglion cell complex thickness in a healthy aging British cohort?

Findings In a cross-sectional twin cohort study of 1657 participants, ganglion cell complex thickness was associated with age, body mass index, and refractive error, as well as estimated glomerular filtration rate. Ganglion cell complex thickness was highly heritable, with additive genetic effects explaining 81% of phenotypic variance.

Meaning Ganglion cell complex thickness appears to be a highly heritable trait and adjustment for age, body mass index, and refractive error is important when using it as a diagnostic parameter.

Methods

Participants

All monozygous and dizygous twin pairs were volunteers in the TwinsUK study, an unselected population-based twin cohort representative of the broader population in terms of disease-associated and lifestyle characteristics.[36] We assessed 1753 individuals for eligibility and included those of European ancestry and who were 18 years or older and excluded 39 not of European ancestry, 33 with treated glaucoma, 4 with ocular hypertension, 7 with other pathologic conditions affecting the retina, 12 with poor-quality SD-OCT images, and 1 with incorrect data. Zygosity was determined by a standardized questionnaire and confirmed using genome-wide genotyping of single-nucleotide polymorphisms or short tandem repeats in some monozygous twin pairs for whom genome-wide data were not available. The study was approved by the Guy's and St. Thomas' ethics committee and all the participants provided written informed consent in accordance with the Declaration of Helsinki.[37]

Clinical Examination

Data were collected between August 27, 2014, and March 31, 2016. Each participant completed a questionnaire to elicit any previous ophthalmic history. Nonophthalmic parameters were assessed, including height, weight, blood pressure, and standard blood markers relevant to microvascular disease (ie, levels of cholesterol, creatinine, glucose, insulin, triglycerides, and urea). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula.[38]

Ophthalmic examination on both eyes included autorefraction, pachymetry, and noncontact measurement of IOP (Visionix120; The Luneau Technology Group). Spherical equivalent (SphE) was calculated using the standard formula of sphere + (cylinder/2). Fundus photography and SD-OCT, including mapping of GCC thickness, were performed (iVue SD-OCT; Optovue).[18,19,39-41] Scans were reviewed by an ophthalmologist (E.B.) and poor-quality scans (n = 59, confirmed by K.M.W.) were excluded from the final analyses. As there was a high correlation between left and right eyes for all measurements (Pearson correlation coefficient, >0.7), we used the mean of the 2 eyes when both eyes were available.

Statistical Analysis

Differences between monozygous and dizygous twins, or between any other group (eg, participants with hypertension or diabetes vs controls), were compared using 2-sample, 2-tailed *t* tests or *z* tests, assuming equal variance. Associations were assessed between GCC thickness and the following 14 factors: age, sex, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), diabetes status, blood pressure (systolic and diastolic), 2 ophthalmic parameters (SphE and IOP), and 6 blood markers (levels of cholesterol, creatinine, glucose, insulin, triglycerides, and urea).

Univariable linear regression analyses were performed, followed by a multivariable linear regression model for factors with an association significant at $P < .05$ in the univariable model. Independent variables were identified using stepwise backward procedure with a threshold for removal set at $P < .05$. Variables that, in the multivariable model, would survive Bonferroni correction for multiple testing ($P \leq .004$; 0.05 divided by 14, where 14 is the number of independent variables tested), were considered associated with GCC thickness. Mean arterial pressure and eGFR are not independent variables, as they are calculated from these variables, so they do not affect the Bonferroni correction threshold. In all regression models, family structure was taken into account.

All analyses, except for the heritability, were carried out using STATA, version 14, statistical package (StataCorp). Heritability analyses were performed using maximum likelihood structural equation twin modeling implemented in the OpenMx package in R (http://openmx.psyc.virginia.edu). We compared the phenotypic variance between monozygous and dizygous twins to estimate the extent to which GCC thickness was a result of additive or dominant genetic effects and common or unique environmental factors. The goodness of fit of the full and reduced additive, common, and unique (ACE) or additive, dominant, and unique (ADE) models was compared with the observed data and the best-fitting model was selected. Before the heritability analysis, GCC thickness was adjusted for age by linear regression of GCC and age and applying the models to the residuals.

Results

Data on GCC thickness were acquired from 3235 eyes of 1657 predominantly female (89.5%) participants, whose demographic and clinical characteristics are presented in **Table 1**. We excluded 39 individuals of non-European ancestry, 44 with ocular pathologic conditions, and 13 for other reasons. The individuals who were excluded for ocular pathologic conditions or other reasons were a mean of 14.3 years older than the remaining participants, who were a mean (SD) age of 56.0 (15.3) years (range, 18-90 years). Monozygous twins were on average younger than the dizygous twins (54 vs 59 years) and had thicker mean GCC (96.4 vs 95.3 µm; $P = .01$). Monozygous and dizygous twins also had different mean (SD) values for the following parameters: BMI (25.6 [0.16] vs 26.5 [0.21]), SphE (−0.21 [0.08] vs 0.15 [0.10] diopters), systolic blood pressure (126.3 [0.58] vs 128.9 [0.66] mm Hg), and serum cholesterol level (206.95 [1.54] vs 212.36 [1.54] mg/dL [to convert to millimoles per liter, multiply by 0.0259]) ($P \leq .01$ for all). After linear age adjustment, there were no differences between monozygous and dizygous twins for these variables.

The mean (SD) inner GCC thickness of the study population was 96.0 (7.6) µm (95% CI, 95.1-96.2), with mean (SD) superior thickness of 95.4 (7.7) µm (95% CI, 95.1-95.7) and inferior thickness of 96.6 (8.0) µm (95% CI, 96.2-96.8). The superior and inferior thicknesses were highly correlated with the mean inner GCC thickness (Pearson correlation coefficient, 0.97 for both) and yielded similar results in all analyses.

Table 1. Demographic and Clinical Characteristics of the Study Population

Characteristic	Value
Age, mean (SD), y	56.0 (15.3)
Height, mean (SD), m	
Women	1.6 (0.1)
Men	1.7 (0.1)
Weight, mean (SD), kg	
Women	68.6 (13.2)
Men	82.6 (16.4)
Body mass index, mean (SD)[a]	26.2 (5.1)
Women	26.1 (4.9)
Men	27.3 (4.5)
Blood pressure, mean (SD), mm Hg	
Systolic	127.3 (17.4)
Diastolic	75.8 (10.6)
Female sex, No. (%)	1483 (89.5)
Diabetes, No. (%)	190 (11.5)
Hypertension, No. (%)	290 (17.5)
Receiving antihypertensive treatment, No. (%)	213 (12.9)
IOP, mean (SD), mm Hg	13.3 (2.6)
SphE, mean (SD), diopters	−0.1 (2.4)
CCT, mean (SD), µm	536.2 (35.6)
Total cholesterol, mean (SD), mg/dL	208.5 (42.5)
Creatinine, mean (SD), mg/dL	0.83 (0.15)
Glucose, mean (SD), mg/dL	86.5 (10.8)
Insulin, mean (SD), µIU/mL	49.1 (32.2)
Total triglycerides, mean (SD), mg/dL	88.5 (53.1)
Urea, mean (SD), mg/dL	32.43 (6.6)

Abbreviations: CCT, central corneal thickness; IOP, intraocular pressure; SphE, spherical equivalent.

SI conversion factors: To convert cholesterol to millimoles per liter, multiply by 0.0259; creatinine to micromoles per liter, multiply by 88.4; glucose to millimoles per liter, multiply by 0.0555; triglycerides to millimoles per liter, multiply by 0.0113; and urea to micromoles per liter, multiply by 59.485.

[a] Calculated as weight in kilograms divided by height in meters squared.

Table 2 summarizes the results from the univariable and multivariable stepwise regression analyses. On univariable analysis, the mean GCC was thinner with advancing age (β [SE], −0.12 [0.02]; $P < .001$), increased BMI (β [SE], −0.19 [0.04]; $P < .001$), and higher systolic (β [SE], −0.06 [0.01]; $P < .001$) and diastolic blood pressures (β [SE], −0.06 [0.02]; $P = .003$). The association with systolic and diastolic blood pressure survived adjustment for hypertension status (β [SE], −0.05 [0.01]; $P < .001$; and β [SE], −0.04 [0.02]; $P = .02$, respectively). Of the ocular parameters, only SphE was associated with GCC thickness on univariable analysis (β [SE], 0.45 [0.09]; $P < .001$), with a thinner GCC associated with a more myopic refraction. Regarding blood parameters, on univariable analysis GCC thickness was negatively associated with an increase in levels of creatinine (β [SE], −2.69 [1.28]; $P = .01$), glucose (β [SE], −0.27 [0.003]; $P = .01$), insulin (β [SE], −0.02 [0.01]; $P = .01$), triglyceride (β [SE], −0.01 [0.004]; $P = .01$), and urea (β [SE], −3.42 [0.90]; $P < .001$) levels (Table 2). In multivariable modeling, the mean inner GCC thickness was independently associated with advancing age (β [SE], −0.14 [0.02]; $P < .001$), SphE (β [SE],

Table 2. Univariable and Multivariable Linear Regression Analysis

Characteristic	β (SE)			
	Univariable	*P* Value	Multivariable	*P* Value
Age	−0.12 (0.02)	<.001	−0.14 (0.02)	<.001
Sex	0.31 (0.77)	.69		
Body mass index	−0.19 (0.04)	<.001	−0.15 (0.04)	.001
Blood pressure				
Systolic	−0.06 (0.01)	<.001		
Diastolic	−0.06 (0.02)	.003		
Diabetes	−0.91 (0.47)	.05		
Spherical equivalent	0.45 (0.09)	<.001	0.70 (0.09)	<.001
Intraocular pressure	0.05 (0.08)	.51		
Total cholesterol	−0.01 (0.01)	.27		
Creatinine	−2.69 (1.28)	.01		
Glucose	−0.27 (0.03)	.01		
Insulin	−0.02 (0.01)	.01		
Total triglycerides	−0.01 (0.004)	.01		
Urea	−3.42 (0.90)	<.001		

0.70 [0.09]; $P < .001$), and increased BMI (β [SE], -0.15 [0.04]; $P = .001$) only; these associations would survive correction for multiple testing (Bonferroni threshold, $P \leq .004$).

Given the possible connection between GCC thickness and both blood pressure[42-44] and kidney function,[45] we explored associated parameters even though they did not survive the multivariable analysis. We first examined if mean arterial pressure, which is considered to be a better marker of organ perfusion,[46] was associated with GCC thickness. The mean arterial pressure in our study was a mean (SD) of 92.9 (12.1) mm Hg. Similar to systolic and diastolic blood pressure, mean arterial pressure was associated with GCC thickness, irrespective of hypertension status (β, -0.06; $P < .001$), but not when adjusted for age (β, -0.03; $P = .13$). Next, we looked at whether GCC thickness was associated with the eGFR. In our study, the mean (SD) eGFR was 82.5 (19.6) mL/min/1.73m^2. Higher eGFR was associated with a thicker GCC, independent of age, hypertension, and diabetes status (β, 0.03; $P = .02$).

Finally, we calculated the heritability of GCC thickness in 1432 twins (426 monozygous and 290 dizygous pairs), as 225 individuals had their twin missing. We found the best-fitting model to be the additive genetic and unique environment (AE) model, in which 81% (95% CI, 78%-84%) of variance in age-adjusted GCC thickness was explained by additive genetic factors and the remaining 19% (95% CI, 16%-22%) by unique environmental factors.

Discussion

Many cross-sectional and longitudinal studies have investigated the effects of age, sex, race/ethnicity, IOP, and axial length on RNFL thickness.[47-54] As GCC incorporates RNFL, the 2 variables are correlated (Pearson correlation coefficient, 0.76 in this sample) and likely share risk factors. However, we set out to examine the associations in a large population-based epidemiologic study of GCC thickness in healthy European individuals and found it to be strongly and independently associated with age, BMI, SphE, and eGFR. Except for eGFR, all the other parameters survived correction for multiple testing.

With respect to age, our results are similar to those previously found for RNFL.[55-57] We found, cross-sectionally, a thinner GCC with older age (a mean decrease of 0.14 µm per year). The effect we found is comparable with that found by Zhang et al[54] in 92 controls (0.17 µm per year) in the Advanced Imaging for Glaucoma Study. In contrast, the association between RNFL and BMI is more variable. Morbid obesity had a significant influence on RNFL, retinal ganglion cells, and choroidal thickness in 1 study,[58] while others suggest that BMI plays a role in RNFL thickness only in men[55] or not at all.[59] The UK Biobank study found a significant negative association between macular thickness and both BMI and male sex,[56] which is in keeping with our study findings of a reduction in GCC thickness of 0.15 µm per 1-U increase in BMI. Owing to the small number of male participants in this study, we were underpowered to detect sex effects; however, the direction of effect was the same in the 2 groups (β, -0.19 and $P < .001$ for women; β, -0.28 and $P = .20$ for men).

Studies of RNFL thickness from the European Prospective Investigation Into Cancer and Nutrition (EPIC)-Norfolk and UK Biobank cohorts found RNFL and macular thickness to be thinner in men than in women.[55,56] Wang et al[60] found no sex differences in the mean thickness of GCC and ganglion cells and the inner plexiform layer in a Chinese cohort. However, while Mwanza et al[61] did not find a difference between the sexes for mean thickness of ganglion cells and the inner plexiform layer ($P = .36$), they did find that male sex was associated with GCC thickness in multivariate analysis with RNFL thickness, age, and axial length (β, -1.62; $P = .005$). We did not observe a difference in mean (SD) GCC thickness between the 174 men (95.7 [7.98] µm) and 1483 women (96.1 [7.45] µm; $P = .69$) in our study.

Of the ophthalmic parameters, GCC thickness was associated only with SphE, with more myopic eyes having a thinner GCC and a mean increase in thickness of 0.70 µm per 1-diopter increase in SphE. Previous studies have proposed that longer axial length is associated with thinner RNFL.[62-64] However, Khawaja et al,[55] using scanning laser polarimetry in the EPIC-Norfolk study, found that longer axial length appeared to be associated with a thicker RNFL. There is some controversy as to the possible magnification effects of the eye with increasing ametropia, which is not routinely corrected for by SD-OCT software.[55,65,66] Some studies using SD-OCT have found that this association reversed once ocular magnification was mathematically corrected.[65,67] Patel et al[56] demonstrated that, on results of SD-OCT, increasing myopia was associated with increased central macular thickness but a decrease in the other macular subfield thicknesses, resulting in an overall positive association with refractive error. Optical coherence tomography also has been shown to be more repeatable and sensitive than glaucoma diagnoses by scanning laser polarimetry at detecting glaucoma in cases of high myopia.[40,68-72] Irrespective of whether the effect is physiological or owing to optics, we propose that future studies of GCC

should consider adjusting for SphE or axial length, given their strong association with GCC thickness.

Although IOP is a known risk factor for POAG, we were not able to identify an association between IOP and GCC thickness in our study. However, this is in agreement with the findings from RNFL association studies, such as the EPIC-Norfolk, UK Biobank, and Advanced Imaging for Glaucoma Study groups.[54-56] It is possible that our exclusion of individuals taking IOP-lowering medication diminished the association with GCC thickness, although we excluded only 37 individuals on this criterion. Furthermore, there is increasing evidence for the role of the association between IOP and intracranial pressure, known as the translaminar pressure difference, in the pathophysiologic cause of glaucoma.[73,74] It may be that the IOP does not damage retinal ganglion cell axons at the lamina cribrosa until it reaches a given threshold, which would, in part, further explain the pathophysiologic cause of normal-tension glaucoma.

Vascular dysregulation has been proposed to play an important role in POAG[29-32] and several recent studies have suggested that systemic hypertension is associated with RNFL and GCC thinning, which may represent an important consideration when using retinal SD-OCT measurements as a diagnostic tool.[42-44] Therefore, in this study, we explored the association between GCC thickness and blood pressure (systolic, diastolic, and mean arterial pressure). We did find GCC to be thinner in individuals with hypertension (94.3 μm) vs those with normal blood pressure (96.3 μm; $P < .001$), and an increase in any of the blood pressure parameters was associated with GCC thickness in univariable analyses. However, this association was small (β, -0.06 μm) and not independent from the effect of age.

Similarly, some studies have shown diabetes to have an early neurodegenerative effect on the retina, causing neuronal dysfunction, leading to thinning of both the RNFL and GCC, with associated functional visual deficits.[75-82] Chhablani et al[79] showed that inner retinal thinning was present in patients with type 2 diabetes, even before signs of retinopathy were visible, while Salvi et al[80] showed a significant association of GCC thinning in patients with diabetic polyneuropathy but not in those with retinopathy. We did not find an association between a diagnosis of diabetes and GCC thickness, nor with fasting serum glucose and insulin levels. The participants in the TwinsUK study cohort are generally healthy, so we may be underpowered to detect effects specific to hypertension or diabetes, although our study did include 290 and 190 participants with these respective diagnoses.

Finally, we found eGFR to be associated with mean GCC thickness. The association survived correction for the effects of age, diabetes, and hypertension, accounting for a 0.03-μm change in GCC thickness per 1-U change in eGFR. This finding suggests that there may be an association between renal function and retinal neuropathy. Srivastav et al[83] found an association between increased serum urea and creatinine levels and decreased RNFL thickness in patients with diabetes, while Shim et al[84] recently demonstrated that low eGFR levels are independently associated with POAG. However, there is a paucity of data regarding this potential association in the absence of diabetes. One study, by Demir et al,[45] evaluated the RNFL thickness in patients with chronic renal failure without diabetes, concluding that it was significantly thinner than normal. Our data are consistent with this notion, but further investigation is required to elucidate the underlying association. It is accepted that the kidneys and retina share common developmental pathways and structural similarities, such as the vascular configuration and type IV collagen basement-membrane composition.[85] Therefore, if renal function correlates with retinal neuronal anatomy in a healthy population, it may become a factor to consider in the interpretation of GCC thickness as a diagnostic parameter.

This is the first study, to our knowledge, that explores the heritability of GCC thickness. We found GCC thickness to be highly heritable, with additive genetic effects accounting for 81% of variance, while unique environmental effects explained the remaining 19% of variance. This finding is comparable with the heritability estimates for central retinal thickness (90%) and RNFL thickness (between 48% and 82%).[86-88] It would be interesting to explore whether any of the known genetic risk factors for POAG and its other endophenotypes also influence GCC thickness. Alleles in *SIX1-SIX6* (GenBank: 6495 and 4990, respectively) have been associated with POAG in genome-wide association studies, and with a decrease in global and sectoral RNFL thickness in individuals of European and Asian descent.[89-91]

Limitations

This study contains some limitations. First, the TwinsUK study cohort is predominantly females of European ancestry, limiting our ability to draw conclusions as to the effect of sex or race/ethnicity on GCC thickness. As this is a cross-sectional study, we cannot draw any conclusions on causation and, as an observational study, we are potentially susceptible to residual confounding and missing data. As we relied on self-reported ocular and systemic disease, as well as drug history, we may be liable to misspecification of variables. Our novel findings require replication.

Conclusions

Ganglion cell complex thickness is a highly heritable trait. We identified an association between SD-OCT-derived GCC thickness measurements and age, refractive error, BMI, and eGFR in a healthy, aging, predominantly female cohort of European descent. The associations between SphE, BMI, and age are strong and therefore adjustment is likely to be relevant if GCC thickness were to be used as a clinical parameter. Future studies of GCC and its association with glaucoma or neurologic disease should take these associations into account. We did not find GCC thickness to be independently associated with sex, diabetes, blood pressure, or IOP. Further studies are required to establish the precise association between renal function and inner retinal structures and to investigate the genetic factors underlying GCC thickness.

ARTICLE INFORMATION

Accepted for Publication: September 28, 2016.

Published Online: November 17, 2016. doi:10.1001/jamaophthalmol.2016.4486

Author Contributions: Dr Bloch and Ms Yonova-Doing had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis. Dr Bloch and Ms Yonova-Doing contributed equally to this work.
Study concept and design: Bloch, Yonova-Doing, Williams, Hammond.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Bloch, Yonova-Doing, Hammond.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Bloch, Yonova-Doing, Jones-Odeh, Williams, Hammond.
Obtained funding: Hammond.
Administrative, technical, or material support: Bloch, Williams, Kozareva.
Study supervision: Hammond.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This study received funding from the International Glaucoma Association. The Wellcome Trust and the European Community's Seventh Framework Programme (FP7/2007-2013) supported the TwinsUK study. The study also received support from the National Institute for Health Research–funded BioResource, Clinical Research Facility, and Biomedical Research Centre based at Guy's and St. Thomas' National Health Service Foundation Trust in partnership with King's College London. Ms Yonova-Doing received funding from the Biotechnology and Biological Sciences Research Council and the London Interdisciplinary Doctoral Program. Ms Williams received funding from the Medical Research Council Clinical Research Training Fellowship.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank all the twin volunteers for their participation.

REFERENCES

1. Varma R, Lee PP, Goldberg I, Kotak S. An assessment of the health and economic burdens of glaucoma. *Am J Ophthalmol*. 2011;152(4):515-522.

2. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90(3):262-267.

3. Quigley HA. Neuronal death in glaucoma. *Prog Retin Eye Res*. 1999;18(1):39-57.

4. McKinnon SJ. Glaucoma, apoptosis, and neuroprotection. *Curr Opin Ophthalmol*. 1997;8(2):28-37.

5. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311(18):1901-1911.

6. Harwerth RS, Carter-Dawson L, Shen F, Smith EL III, Crawford ML. Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1999;40(10):2242-2250.

7. Cordeiro MF, Guo L, Luong V, et al. Real-time imaging of single nerve cell apoptosis in retinal neurodegeneration. *Proc Natl Acad Sci U S A*. 2004;101(36):13352-13356.

8. Balendra SI, Normando EM, Bloom PA, Cordeiro MF. Advances in retinal ganglion cell imaging. *Eye (Lond)*. 2015;29(10):1260-1269.

9. Tatham AJ, Weinreb RN, Zangwill LM, Liebmann JM, Girkin CA, Medeiros FA. The relationship between cup-to-disc ratio and estimated number of retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2013;54(5):3205-3214.

10. Wang M, Hood DC, Cho JS, et al. Measurement of local retinal ganglion cell layer thickness in patients with glaucoma using frequency-domain optical coherence tomography [published correction appears in *Arch Ophthalmol*. 2010;128(9):1150]. *Arch Ophthalmol*. 2009;127(7):875-881.

11. Ishikawa H, Stein DM, Wollstein G, Beaton S, Fujimoto JG, Schuman JS. Macular segmentation with optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2005;46(6):2012-2017.

12. Chen TC, Cense B, Pierce MC, et al. Spectral domain optical coherence tomography: ultra-high speed, ultra-high resolution ophthalmic imaging. *Arch Ophthalmol*. 2005;123(12):1715-1720.

13. Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol*. 1990;300(1):5-25.

14. Hood DC, Raza AS, de Moraes CG, Liebmann JM, Ritch R. Glaucomatous damage of the macula. *Prog Retin Eye Res*. 2013;32:1-21.

15. Yang Z, Tatham AJ, Weinreb RN, Medeiros FA, Liu T, Zangwill LM. Diagnostic ability of macular ganglion cell inner plexiform layer measurements in glaucoma using swept source and spectral domain optical coherence tomography. *PLoS One*. 2015;10(5):e0125957.

16. Tan O, Chopra V, Lu AT, et al. Detection of macular ganglion cell loss in glaucoma by Fourier-domain optical coherence tomography. *Ophthalmology*. 2009;116(12):2305-14.e1, 2.

17. Kim NR, Lee ES, Seong GJ, Kim JH, An HG, Kim CY. Structure-function relationship and diagnostic value of macular ganglion cell complex measurement using Fourier-domain OCT in glaucoma. *Invest Ophthalmol Vis Sci*. 2010;51(9):4646-4651.

18. Schulze A, Lamparter J, Pfeiffer N, Berisha F, Schmidtmann I, Hoffmann EM. Diagnostic ability of retinal ganglion cell complex, retinal nerve fiber layer, and optic nerve head measurements by Fourier-domain optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol*. 2011;249(7):1039-1045.

19. Garas A, Vargha P, Holló G. Diagnostic accuracy of nerve fibre layer, macular thickness and optic disc measurements made with the RTVue-100 optical coherence tomograph to detect glaucoma. *Eye (Lond)*. 2011;25(1):57-65.

20. Medeiros FA, Zangwill LM, Bowd C, Vessani RM, Susanna R Jr, Weinreb RN. Evaluation of retinal nerve fiber layer, optic nerve head, and macular thickness measurements for glaucoma detection using optical coherence tomography. *Am J Ophthalmol*. 2005;139(1):44-55.

21. Tan O, Li G, Lu AT, Varma R, Huang D; Advanced Imaging for Glaucoma Study Group. Mapping of macular substructures with optical coherence tomography for glaucoma diagnosis. *Ophthalmology*. 2008;115(6):949-956.

22. Jeoung JW, Choi YJ, Park KH, Kim DM. Macular ganglion cell imaging study: glaucoma diagnostic accuracy of spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013;54(7):4422-4429.

23. Mwanza JC, Durbin MK, Budenz DL, et al. Glaucoma diagnostic accuracy of ganglion cell-inner plexiform layer thickness: comparison with nerve fiber layer and optic nerve head. *Ophthalmology*. 2012;119(6):1151-1158.

24. Oddone F, Lucenteforte E, Michelessi M, et al. Macular versus retinal nerve fiber layer parameters for diagnosing manifest glaucoma: a systematic review of diagnostic accuracy studies. *Ophthalmology*. 2016;123(5):939-949.

25. Loewen NA, Zhang X, Tan O, et al; Advanced Imaging for Glaucoma Study Group. Combining measurements from three anatomical areas for glaucoma diagnosis using Fourier-domain optical coherence tomography. *Br J Ophthalmol*. 2015;99(9):1224-1229.

26. Nouri-Mahdavi K, Nowroozizadeh S, Nassiri N, et al. Macular ganglion cell/inner plexiform layer measurements by spectral domain optical coherence tomography for detection of early glaucoma and comparison to retinal nerve fiber layer measurements. *Am J Ophthalmol*. 2013;156(6):1297-1307.e2.

27. Meira-Freitas D, Lisboa R, Tatham A, et al. Predicting progression in glaucoma suspects with longitudinal estimates of retinal ganglion cell counts. *Invest Ophthalmol Vis Sci*. 2013;54(6):4174-4183.

28. Coleman AL, Miglior S. Risk factors for glaucoma onset and progression. *Surv Ophthalmol*. 2008;53(suppl 1):S3-S10.

29. Osborne NN, Melena J, Chidlow G, Wood JP. A hypothesis to explain ganglion cell death caused by vascular insults at the optic nerve head: possible implication for the treatment of glaucoma. *Br J Ophthalmol*. 2001;85(10):1252-1259.

30. Moore D, Harris A, Wudunn D, Kheradiya N, Siesky B. Dysfunctional regulation of ocular blood flow: a risk factor for glaucoma? *Clin Ophthalmol*. 2008;2(4):849-861.

31. Hayreh SS. The role of age and cardiovascular disease in glaucomatous optic neuropathy. *Surv Ophthalmol*. 1999;43(suppl 1):S27-S42.

32. Lawrenson JG. Histopathology and pathogenesis of glaucomatous optic neuropathy. In: Edgar DF, Rudnicka AR, eds. *Glaucoma Identification and Co-management*. Philadelphia, PA: Elsevier Limited; 2007:27-35.

33. Zhou M, Wang W, Huang W, Zhang X. Diabetes mellitus as a risk factor for open-angle glaucoma: a systematic review and meta-analysis. *PLoS One*. 2014;9(8):e102972.

34. Charlesworth J, Kramer PL, Dyer T, et al. The path to open-angle glaucoma gene discovery: endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. *Invest Ophthalmol Vis Sci*. 2010;51(7):3509-3514.

35. Abu-Amero K, Kondkar AA, Chalam KV. An updated review on the genetics of primary open angle glaucoma. *Int J Mol Sci*. 2015;16(12):28886-28911.

36. Moayyeri A, Hammond CJ, Valdes AM, Spector TD. Cohort Profile: TwinsUK and healthy ageing twin study. *Int J Epidemiol*. 2013;42(1):76-85.

37. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

38. Levey AS, Coresh J, Greene T, et al; Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 2006;145(4):247-254.

39. Mori S, Hangai M, Sakamoto A, Yoshimura N. Spectral-domain optical coherence tomography measurement of macular volume for diagnosing glaucoma. *J Glaucoma*. 2010;19(8):528-534.

40. Bertuzzi F, Benatti E, Esempio G, Rulli E, Miglior S. Evaluation of retinal nerve fiber layer thickness measurements for glaucoma detection: GDx ECC versus spectral-domain OCT. *J Glaucoma*. 2014;23(4):232-239.

41. Takagi ST, Kita Y, Takeyama A, Tomita G. Macular retinal ganglion cell complex thickness and its relationship to the optic nerve head topography in glaucomatous eyes with hemifield defects. *J Ophthalmol*. 2011;2011:914250.

42. Gangwani RA, Lee JW, Mo HY, et al. The correlation of retinal nerve fiber layer thickness with blood pressure in a Chinese hypertensive population. *Medicine (Baltimore)*. 2015;94(23):e947.

43. Akay F, Gündoğan FC, Yolcu U, Toyran S, Tunç E, Uzun S. Retinal structural changes in systemic arterial hypertension: an OCT study. *Eur J Ophthalmol*. 2016;26(5):436-441.

44. Sahin OZ, Sahin SB, Ayaz T, et al. The impact of hypertension on retinal nerve fiber layer thickness and its association with carotid intima media thickness. *Blood Press*. 2015;24(3):178-184.

45. Demir MN, Eksioglu U, Altay M, et al. Retinal nerve fiber layer thickness in chronic renal failure without diabetes mellitus. *Eur J Ophthalmol*. 2009;19(6):1034-1038.

46. Brzezinski WA. Blood pressure. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical and Laboratory Examination*. 3rd ed. Boston, MA: Butterworths; 1990:95-97.

47. Zhao L, Wang Y, Chen CX, Xu L, Jonas JB. Retinal nerve fibre layer thickness measured by Spectralis spectral-domain optical coherence tomography: the Beijing Eye Study. *Acta Ophthalmol*. 2014;92(1):e35-e41.

48. Patel NB, Lim M, Gajjar A, Evans KB, Harwerth RS. Age-associated changes in the retinal nerve fiber layer and optic nerve head. *Invest Ophthalmol Vis Sci*. 2014;55(8):5134-5143.

49. Girkin CA, McGwin G Jr, Sinai MJ, et al. Variation in optic nerve and macular structure with age and race with spectral-domain optical coherence tomography. *Ophthalmology*. 2011;118(12):2403-2408.

50. Budenz DL, Anderson DR, Varma R, et al. Determinants of normal retinal nerve fiber layer thickness measured by Stratus OCT. *Ophthalmology*. 2007;114(6):1046-1052.

51. Leung CK, Yu M, Weinreb RN, et al. Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography: a prospective analysis of age-related loss. *Ophthalmology*. 2012;119(4):731-737.

52. Leung CK, Ye C, Weinreb RN, Yu M, Lai G, Lam DS. Impact of age-related change of retinal nerve fiber layer and macular thicknesses on evaluation of glaucoma progression. *Ophthalmology*. 2013;120(12):2485-2492.

53. Ueda K, Kanamori A, Akashi A, Tomioka M, Kawaka Y, Nakamura M. Effects of axial length and age on circumpapillary retinal nerve fiber layer and inner macular parameters measured by 3 types of SD-OCT instruments. *J Glaucoma*. 2016;25(4):383-389.

54. Zhang X, Francis BA, Dastiridou A, et al; Advanced Imaging for Glaucoma Study Group. Longitudinal and cross-sectional analyses of age effects on retinal nerve fiber layer and ganglion cell complex thickness by Fourier-Domain OCT. *Transl Vis Sci Technol*. 2016;5(2):1.

55. Khawaja AP, Chan MP, Garway-Heath DF, et al. Associations with retinal nerve fiber layer measures in the EPIC-Norfolk Eye Study. *Invest Ophthalmol Vis Sci*. 2013;54(7):5028-5034.

56. Patel PJ, Foster PJ, Grossi CM, et al; UK Biobank Eyes and Vision Consortium. Spectral-domain optical coherence tomography imaging in 67 321 adults: associations with macular thickness in the UK Biobank study. *Ophthalmology*. 2016;123(4):829-840.

57. Knight OJ, Girkin CA, Budenz DL, Durbin MK, Feuer WJ; Cirrus OCT Normative Database Study Group. Effect of race, age, and axial length on optic nerve head parameters and retinal nerve fiber layer thickness measured by Cirrus HD-OCT. *Arch Ophthalmol*. 2012;130(3):312-318.

58. Dogan B, Kazim Erol M, Dogan U, et al. The retinal nerve fiber layer, choroidal thickness, and central macular thickness in morbid obesity: an evaluation using spectral-domain optical coherence tomography. *Eur Rev Med Pharmacol Sci*. 2016;20(5):886-891.

59. Zhu BD, Li SM, Li H, et al; Anyang Childhood Eye Study Group. Retinal nerve fiber layer thickness in a population of 12-year-old children in central China measured by iVue-100 spectral-domain optical coherence tomography: the Anyang Childhood Eye Study. *Invest Ophthalmol Vis Sci*. 2013;54(13):8104-8111.

60. Wang J, Gao X, Huang W, et al. Swept-source optical coherence tomography imaging of macular retinal and choroidal structures in healthy eyes. *BMC Ophthalmol*. 2015;15(15):122.

61. Mwanza JC, Durbin MK, Budenz DL, et al; Cirrus OCT Normative Database Study Group. Profile and predictors of normal ganglion cell–inner plexiform layer thickness measured with frequency-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52(11):7872-7879.

62. Wang YX, Pan Z, Zhao L, You QS, Xu L, Jonas JB. Retinal nerve fiber layer thickness: the Beijing Eye Study 2011. *PLoS One*. 2013;8(6):e66763.

63. Zhao JJ, Zhuang WJ, Yang XQ, Li SS, Xiang W. Peripapillary retinal nerve fiber layer thickness distribution in Chinese with myopia measured by 3D-optical coherence tomography. *Int J Ophthalmol*. 2013;6(5):626-631.

64. Alasil T, Wang K, Keane PA, et al. Analysis of normal retinal nerve fiber layer thickness by age, sex, and race using spectral domain optical coherence tomography. *J Glaucoma*. 2013;22(7):532-541.

65. Kang SH, Hong SW, Im SK, Lee SH, Ahn MD. Effect of myopia on the thickness of the retinal nerve fiber layer measured by Cirrus HD optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51(8):4075-4083.

66. Weinreb RN. Evaluating the retinal nerve fiber layer in glaucoma with scanning laser polarimetry. *Arch Ophthalmol*. 1999;117(10):1403-1406.

67. Savini G, Barboni P, Parisi V, Carbonelli M. The influence of axial length on retinal nerve fibre layer thickness and optic-disc size measurements by spectral-domain OCT. *Br J Ophthalmol*. 2012;96(1):57-61.

68. Garas A, Tóth M, Vargha P, Holló G. Comparison of repeatability of retinal nerve fiber layer thickness measurement made using the RTVue Fourier-domain optical coherence tomograph and the GDx scanning laser polarimeter with variable or enhanced corneal compensation. *J Glaucoma*. 2010;19(6):412-417.

69. Michelessi M, Lucenteforte E, Oddone F, et al. Optic nerve head and fibre layer imaging for diagnosing glaucoma. *Cochrane Database Syst Rev*. 2015;11(11):CD008803.

70. Choi YJ, Jeoung JW, Park KH, Kim DM. Glaucoma detection ability of ganglion cell–inner plexiform layer thickness by spectral-domain optical coherence tomography in high myopia. *Invest Ophthalmol Vis Sci*. 2013;54(3):2296-2304.

71. Shoji T, Sato H, Ishida M, Takeuchi M, Chihara E. Assessment of glaucomatous changes in subjects with high myopia using spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52(2):1098-1102.

72. Kim NR, Lee ES, Seong GJ, et al. Comparing the ganglion cell complex and retinal nerve fibre layer measurements by Fourier domain OCT to detect glaucoma in high myopia. *Br J Ophthalmol*. 2011;95(8):1115-1121.

73. Jonas JB. Role of cerebrospinal fluid pressure in the pathogenesis of glaucoma. *Acta Ophthalmol*. 2011;89(6):505-514.

74. Siaudvytyte L, Januleviciene I, Daveckaite A, et al. Literature review and meta-analysis of translaminar pressure difference in open-angle glaucoma. *Eye (Lond)*. 2015;29(10):1242-1250.

75. Skarf B. Retinal nerve fibre layer loss in diabetes mellitus without retinopathy. *Br J Ophthalmol*. 2002;86(7):709.

76. van Dijk HW, Verbraak FD, Kok PH, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci*. 2010;51(7):3660-3665.

77. van Dijk HW, Verbraak FD, Stehouwer M, et al. Association of visual function and ganglion cell layer

thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. *Vision Res*. 2011;51(2):224-228.

78. Demir M, Oba E, Sensoz H, Ozdal E. Retinal nerve fiber layer and ganglion cell complex thickness in patients with type 2 diabetes mellitus. *Indian J Ophthalmol*. 2014;62(6):719-720.

79. Chhablani J, Sharma A, Goud A, et al. Neurodegeneration in type 2 diabetes: evidence from spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2015;56(11):6333-6338.

80. Salvi L, Plateroti P, Balducci S, et al. Abnormalities of retinal ganglion cell complex at optical coherence tomography in patients with type 2 diabetes: a sign of diabetic polyneuropathy, not retinopathy. *J Diabetes Complications*. 2016;30(3):469-476.

81. Barber AJ. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003;27(2):283-290.

82. Kern TS, Barber AJ. Retinal ganglion cells in diabetes. *J Physiol*. 2008;586(18):4401-4408.

83. Srivastav K, Saxena S, Mahdi AA, Kruzliak P, Khanna VK. Increased serum urea and creatinine levels correlate with decreased retinal nerve fibre layer thickness in diabetic retinopathy. *Biomarkers*. 2015;20(6-7):470-473.

84. Shim SH, Sung KC, Kim JM, et al; Epidemiologic Survey Committee of the Korean Ophthalmological Society. Association between renal function and open-angle glaucoma: the Korea National Health and Nutrition Examination Survey 2010-2011. *Ophthalmology*. 2016;123(9):1981-1988.

85. Savige J, Ratnaike S, Colville D. Retinal abnormalities characteristic of inherited renal disease. *J Am Soc Nephrol*. 2011;22(8):1403-1415.

86. Chamberlain MD, Guymer RH, Dirani M, Hopper JL, Baird PN. Heritability of macular thickness determined by optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2006;47(1):336-340.

87. van Koolwijk LM, Despriet DD, van Duijn CM, et al. Genetic contributions to glaucoma: heritability of intraocular pressure, retinal nerve fiber layer thickness, and optic disc morphology. *Invest Ophthalmol Vis Sci*. 2007;48(8):3669-3676.

88. Hougaard JL, Kessel L, Sander B, Kyvik KO, Sørensen TI, Larsen M. Evaluation of heredity as a determinant of retinal nerve fiber layer thickness as measured by optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2003;44(7):3011-3016.

89. Kuo JZ, Zangwill LM, Medeiros FA, et al. Quantitative trait locus analysis of SIX1-SIX6 with retinal nerve fiber layer thickness in individuals of European descent. *Am J Ophthalmol*. 2015;160(1):123-30.e1.

90. Carnes MU, Liu YP, Allingham RR, et al; NEIGHBORHOOD Consortium Investigators. Discovery and functional annotation of *SIX6* variants in primary open-angle glaucoma. *PLoS Genet*. 2014;10(5):e1004372.

91. Cheng CY, Allingham RR, Aung T, et al. Association of common *SIX6* polymorphisms with peripapillary retinal nerve fiber layer thickness: the Singapore Chinese Eye Study. *Invest Ophthalmol Vis Sci*. 2014;56(1):478-483.